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DRC-6749--AN AVIAN WETTING AGENT OF LOW AQUATIC TOXICITY

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ABSTRACT

PA-14, the compound presently registered for lethal control of blackbirds and starlings at roosts, is labelled for use only at upland sites. Research was undertaken to identify another wetting agent which might be registered for use at aquatic sites. Of 90 samples received from suppliers, 60 were evaluated for wetting ability in a standard laboratory test. Of these, 10 were chosen for test against an aquatic indicator animal, *Daphnia*. One of the 10, DRC-6749, a block copolymer, was found significantly low in toxicity, and underwent further aquatic testing against 6 fish and 1 mussel species. Under standard test conditions, no fish mortality occurred at concentrations up to 2,000 mg/l. Some fish mortality occurred at higher temperatures: LC₅₀'s for rainbow trout at 17°C and bluegills at 22°C were 801 and 740 mg/l respectively. No mussel mortality occurred at these temperatures with concentrations up to 2,000 mg/l. Laboratory spray tests showed that DRC-6749 solutions were capable of wetting birds at reasonable application rates, and a field trial confirmed this with an estimated kill of 63,200 blackbirds and starlings from an application of 208 l of DRC-6749 and 2.3 cm of water over a 0.46 ha area.

INTRODUCTION

Federal animal damage control researchers have been involved in developing wetting agents for lethal control of roosting blackbirds and starlings since 1958 (D. L. Campbell pers. com-

mun.). In early field trials of the concept, various terrestrial application methods or aerial tankers were used to deliver the wetting agents to the birds. Further development led to the use of agricultural spray aircraft for low-volume applications (Lefebvre and Seubert 1970). One wetting agent (DRC-6642) was registered by the Environmental Protection Agency (EPA) in 1974 as Compound PA-14 Avian Stressing Agent, and the low volume aerial technique was subsequently used to treat roosts in Kentucky and Tennessee (Garner 1978). Operational uses of the method met with varying degrees of success, with failures occurring when anticipated necessary rainfall and/or cold temperatures did not occur following surfactant application. Recent development has led to modification of the EPA registration to permit use of ground-based application systems to apply PA-14 and water, and obviate the need for rainfall (Stickley et al. 1986).

Although the new application system enhances the likelihood of successful roost population reduction, its operational use with PA-14 is limited to upland sites because of the wetting agent's toxicity to aquatic animals, a characteristic it shares with many surfactants (Abel 1974, Margaritis and Creese 1978). Laboratory tests showed that the 96-h median lethal concentration (LC₅₀, or calculated level which would kill half of an exposed population) of PA-14 ranged from 1.4 to 250 mg/l for several invertebrate taxa (Marking and Chandler 1981) and 3 to 7 mg/l for 2 fish species (Lefebvre and Seubert 1970). On an aquatic acute toxicity scale, PA-14 thus would be rated "Moderately Toxic" (U. S. Fish and Wildlife Service (USFWS) 1984). Recent research (Mott et al. unpubl. data) has shown that most roosts in the Kentucky-Tennessee area, despite being associated with water to various degrees (Garner 1978), are treatable with

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PA-14 because roost soils adsorb much of the chemical, and filter dams of combined straw and ground clay can be used to remove from 55 to 89% of the PA-14 that does run off. Nevertheless, even in this region, there are many sites where use of a compound less toxic to aquatic life would be desirable, and in other areas where roosts are more likely to occur near or over water (North Dakota, Louisiana, and Texas), use of a more innocuous wetting agent would be essential. In light of this, research was undertaken to identify an efficient feather-wetting agent of lower aquatic toxicity in order to expand the utility of the wetting agent concept in blackbird-starling roost management.

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METHODS

Initial Screening

Suppliers of soaps and synthetic surfactants were identified through searches of the Thomas Register (Thomas Publ. Co. 1984), and files of the Florida Field Station (FFS) of the Denver Wildlife Research Center (DWRC). Requests for samples were sent to approximately 100 manufacturers and formulators, and approximately 90 were received. From these, and from previously-obtained stocks maintained at the FFS, 60 candidates were chosen for evaluation of their wetting ability. The selections were made on the basis of water solubility and suppliers' descriptions of wetting characteristics.

The Draves-Clarkson Cotton Skein Test (Amer. Assoc. Textile Chem. Colorists 1969) was used to evaluate the selected surfactants for wetting efficiency.

This test measures the time taken for a weighted standardized skein of cotton thread to begin sinking after being placed in a cylinder of aqueous surfactant solution. Of those tested, 36 produced sinking times of ≤ 5.0 sec at concentrations $\leq 0.5\%$. From this group, 9 compounds, representing several chemical classes, were chosen to undergo the first stage of toxicity testing. PA-14 also was included for comparison.

Aquatic Toxicity

Daphnia screen -- The 10 surfactants chosen for toxicity testing were bioassayed against Daphnia magna at the USFWS National Fishery Research Center, LaCrosse, Wisconsin. Daphnia is a sensitive indicator of surfactant toxicity to aquatic invertebrates and fish (Lewis and Suprenant 1983, Maki 1979).

Daphnia were cultured according to methods of the EPA (1984). Animals less than 24-h old were exposed to each concentration in duplicate groups of 10. Exposures were in reconstituted water (44 mg/l as CaCO_3 hardness) maintained at 22°C . Preliminary range-finding tests consisted of 3 concentrations and an untreated control; definitive tests involved 9 concentrations and an untreated control.

Toxicity was described by EC_{50} (concentration calculated to produce 50% immobility in 48 h) and 95% confidence intervals calculated by the method of Litchfield and Wilcoxon (1949).

Fish and mollusc tests -- One of the 10 surfactants tested against Daphnia, DRC-6749, also was tested against 6 species of fish and 1 species of mussel. Species of the test animals are presented in Table 1. Fish were cultured according to the methods of Hunn et al. (1968).

The mussel was the invertebrate of choice for these tests because of the large number of endangered mussels endemic to the river systems of Kentucky and Tennessee (Parker and Dixon 1980) where the use of wetting agents at bird roosts is expected to be heaviest. Mussels were taken from the wild, and held in laboratory tanks for 2 weeks for acclimation and determination of health prior to testing.

Table 1. Toxicity of DRC-6749, PA-14, ethanol and methanol to selected aquatic animals under standard conditions¹.

Species	96-h LC ₅₀ (mg/l)			
	DRC-6749	PA-14	ethanol ²	methanol ²
Rainbow trout (<u>Salmo gairdneri</u>)	>2,000	3.3 (2.8-3.9)	13,000 (12,000-16,000)	19,000 (18,000-20,000)
White sucker (<u>Catostomus commersoni</u>)	>2,000	--	--	--
Fathead minnow (<u>Pimephales promelas</u>)	>2,000	--	--	--
Channel catfish (<u>Ictalurus punctatus</u>)	>2,000	5.5 (5.0-6.0)	--	--
Bluegill (<u>Lepomis macrochirus</u>)	>2,000	4.7 (4.0-5.5)	--	--
Yellow perch (<u>Perca flavescens</u>)	>2,000	--	--	--
Fawnfoot mussel (<u>Truncilla donaciformis</u>)	>2,000	--	--	--
River horn snail (<u>Oxytrema catenaria</u>)	--	6.0 (4.1-8.7)	--	--
Asiatic clam (<u>Corbicula manilensis</u>)	--	250 (210-290)	--	--

¹ Standard water conditions (CMTT 1975): 12°C, pH 7.2-7.6, hardness 40-48 mg/l as CaCO₃, alkalinity 30-35 mg/l as CaCO₃. Hardness for PA-14 mollusc tests was 24 mg/l (Marking and Chandler 1981).

² Mayer and Eilersieck (1986) Units shown are µl/l.

Acute toxicity tests were conducted in accordance with the methods outlined by ASTM Committee E-35 on Pesticides (1980) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (CMTT) (1975). All species were tested under standard conditions, and the effects of water hardness, pH, and temperature were determined on bluegills, fathead minnows, and rainbow trout. Lighting was on a 12 h light, 12 h dark cycle. Exposures were made in duplicate, with a total of 20 organisms at each concentration. Test concentrations were calculated on the basis of the formulation as supplied. Test conditions and water quality characteristics for the fish tests are

listed in Table 2. Mussels were tested only in soft water at 12 and 17°C. Observations on survival/mortality were recorded at 1, 3, and 6 h during the first day and daily thereafter. LC₅₀ values and 95% confidence intervals were calculated according to the method of Litchfield and Wilcoxon (1949).

Laboratory Bird-wetting Tests

Spray applications of DRC-6749 were made at the FFS to confirm feather-wetting effectiveness. Male red-winged blackbirds (Agelaius phoeniceus) and common grackles (Quiscalus quiscula) were individually caged in 20 x 20 x 20 cm cages and sprayed with solutions of PA-14 and DRC-6749. Solution concentrations of 1 to 20%, at spray rates of

Table 2. Toxicity of DRC-6749 to bluegills, fathead minnows, rainbow trout, and fawnfoot mussels under selected conditions.

Temperature (°C ± 1)	pH (range)	Hardness ¹ (range)	Alkalinity ² (range)	96-hour LC50 (mg/l) and 95% confidence interval			
				Bluegill (95% C.I.)	Fathead minnow	Rainbow trout (95% C.I.)	Fawnfoot mussel
7	7.4 (7.2-7.6)	44 (40-48) soft	32.5 (30-35)	--	--	>2,000	--
12 ³				>2,000	>2,000	>2,000	>2,000
17				>2,000	>2,000	801 (729-880)	>2,000
22				740 (626-875)	>2,000	--	--
12	8.2 (8.0-8.4)	11.5 (10-13) very soft	235 (225-245)	>2,000	>2,000	>2,000	--
12		44 (40-48) soft		>2,000	>2,000	>2,000	--
12		170 (160-180) hard		>2,000	>2,000	>2,000	--
12		300 (280-320) very hard		>2,000	>2,000	>2,000	--
12	6.5 (6.4-6.6)	44 (40-48) soft	32.5 (30-35)	>2,000	>2,000	>2,000	--
12	8.5 (8.4-8.6)			>2,000	>2,000	>2,000	--
12	9.5 (9.4-9.6)			>2,000	>2,000	>2,000	--

¹ Expressed as mg/l CaCO₃ divalent cation

² Expressed as mg/l CaCO₃ buffering capacity

³ Water characteristics on this line are those used in standard acute toxicity tests.

470 to 56,100 l/ha, were applied through TeeJet Even Flat Spray nozzles (Spraying Systems Co., Wheaton, Illinois) mounted over a steel conveyor belt spray table. Birds were sprayed in replicate groups of 4 or 5 and plac-

ed in a cold (0-4.4°C) environmental chamber within 3-5 min. Some groups received additional spray applications of 0.25 to 0.76 cm of water after treatment with wetting agent. One series was resprayed with water after

a period of cold exposure, then returned to the cold chamber.

Solutions for these tests were prepared from a 50% stock solution of DRC-6749 in 95% ethanol; this solution was diluted with water to prepare final treatment concentrations. The alcohol solution mixed with water much more readily than pure DRC-6749, especially at high concentrations, at which what appeared to be spontaneous gelling was a problem.

Field Trial

DRC-6749 was applied to a blackbird-starling roost in a 0.27 ha white pine (*Pinus strobus*) planting in London, Kentucky on the night of 21-22 February 1986. Generally, techniques followed those of Stickley et al. (1986), except that the usual venturi eductor was not employed to introduce the wetting agent into the water stream. The number of sprinklers (7) needed to provide coverage of the small roost area was not large enough to provide enough flow for eductor operation. Instead, DRC-6749 was first formulated as a 50% 2-propanol solution. A total of 416 l of this solution was poured in 19 l increments into a 5,680 l tank being supplied with water to feed a fire department pumper truck. During 107 min (1956-2143) of this mixing, 46,177 l of water, in addition to the 416 l of DRC-6749 solution (451 l actual/ha), was pumped by the fire engine through the system's 7 sprinklers to cover a total area of 0.46 ha. The overall percentage of DRC-6749 to water during the period of wetting agent application was 0.45%. The actual percentage being applied at any given time could have varied substantially from this figure, however, because of the mixing method used. Pumping of water alone continued until 0005, by which time 107,116 l, or 2.3 cm, had been applied. During the treatment, skies were overcast and temperatures were $\geq 1.7 \leq 4.4^\circ\text{C}$. The overnight low was 1.7°C .

RESULTS

Aquatic Toxicity

Daphnia screen -- Only 1 of the candidates, DRC-6749, was lower enough in toxicity to Daphnia than PA-14 (Table

3) to warrant further testing.

Fish and mollusc tests -- DRC-6749 was considerably less toxic to fish than to Daphnia. In 96 h exposures in soft water at 12°C , no mortality occurred with any of the species tested at concentrations up to 2,000 mg/l. Water hardness and pH did not affect the toxicity to rainbow trout, fathead minnows, or bluegills at these concentrations. An increase in toxicity was noted at warmer water temperatures. With rainbow trout, at a temperature of 17°C , the 96-h LC_{50} was 801.0 mg/l; however, no fish were killed at temperatures of 7 or 12°C at concentrations up to 2,000 mg/l. Similarly, the compound was more toxic to bluegills in warmer waters. The 96-h LC_{50} was 740.0 mg/l in 22°C water, but no fish died at water temperatures of 12 or 17°C at concentrations up to 2,000 mg/l. No mortality to mussels occurred at either 12 or 17°C at concentrations up to 2,000 mg/l in 96-h exposures. Results of all the tests in this series are presented in Table 2.

Based on these findings, the relative acute aquatic toxicity of DRC-6749 would be expressed as "Practically Non-toxic" to "Relatively Harmless" (USFWS 1984), depending on species and temperature.

Laboratory bird-wetting tests

Laboratory tests of bird-wetting capabilities showed that DRC-6749, while not as efficient as PA-14, is capable of wetting birds at reasonable application rates (Tables 4-8). Additional testing will be necessary to precisely define optimum concentrations and rates, but the following tentative conclusions were reached:

- Solutions made from DRC-6749/ethanol stock solutions seem to be approximately as effective as solutions made with DRC-6749 and water (Tables 4 and 5).

- At equivalent low concentrations (1%), approximately twice as much DRC-6749 may be required to achieve the same mortality level as PA-14 (Table 5).

- Applications at higher concentrations (10%), followed by water applications, may require considerably more

Table 3. Toxicity of 10 surfactants to Daphnia magna in soft water at 22°C.

Compound	Chemical Type	Concentration (%) ¹	48-h EC ₅₀ ² (mg/l) (95% C.I.)
DRC-6642 (PA-14)	ethoxylated secondary alcohols	100	4.15 (3.05-5.65)
DRC-6749	methyloxirane-oxirane polymer	100	229 (185-284)
DRC-6770	alkyl sulfate	90-96	2.32 (1.59-3.38)
DRC-6771	soap (from coconut oil)	35-37	26.4 (20.6-33.8)
DRC-6777	soap (from soybean and cottonseed oils)	95	7.50 (5.92-9.50)
DRC-6772	alkanolomide sulfosuccinate ester, disodium salt	40	27.4 (22.5-33.3)
DRC-6773	imidazoline dicarboxylic acid	38	43.9 (37.2-51.8)
DRC-6774	sulfonated aliphatic polyester	70	16.0 (13.0-19.7)
DRC-6775	ethoxylated alcohols	100	4.10 (3.40-4.94)
DRC-6776	alkylaryl polyether alcohol	100	9.55 (7.18-12.7)

¹ Concentrations as supplied by manufacturer/formulator.

² Concentration calculated to be effective for producing 50% immobility in 48 h. Not corrected for concentration of surfactant as supplied.

DRC-6749 than PA-14 to achieve equivalent mortality (Tables 6 and 7).

- Further increasing concentration (20%) of DRC-6749 may further decrease effectiveness (Table 8).

- At a given concentration, application of more water after DRC-6749 application may increase effectiveness.

Field Trial

By 2130, birds were beginning to fall from the trees. By the time the operation ended at 0005, many were dead. The next morning an estimated 39,800 birds left the roost.

Dead birds were counted in 41 randomly-selected 0.84 m² plots in the

2,508 m² kill area, a 1.7% sample. The data produced an estimated kill of 63,200 with 95% confidence limits 41,700 to 84,700. Based on the mean, the kill amounted to 252,000 birds/ha. The total roost population prior to spraying was estimated at 103,000 (63,200 dead + 39,800 leaving), or 381,500 birds/ha. The kill thus amounted to approximately 61% of the roost population. Species composition of the kill is presented in Table 9.

DISCUSSION

DRC-6749 belongs to a group of chemicals known industrially as block co-

Table 4. Effects of cold temperature (3.3°C) on male common grackles sprayed with aqueous 1% v/v DRC-6749 solutions.

Solution	Rate (l/ha)	Mortality (deaths/N)
Control (water only)	37,400	0/4
1% DRC-6749	18,700 37,400	0/4 2/4
1% DRC-6749/ETOH ¹	18,700 37,400	2/4 4/4

¹ From stock solution containing 10% v/v DRC-6749 in 95% ethanol.

Table 5. Effect of cold temperatures on male red-winged blackbirds sprayed with aqueous wetting agent solutions.

Solution ¹	Temperature (°C)	Rate (l/ha)	Mortality (deaths/N)
control (water only)	0-1.1	37,400	0/5
	1.1-3.3	56,100	0/4
0.5% PA-14	0-1.1	9,300	0/5
	0-1.1	18,700	4/5
1.0% PA-14	0-1	9,300	0/5
	0-3.3	18,700	7/9
	1.7-3.3	37,400	4/4
	1.7-3.3	56,100	4/4
1.0% DRC-6749	1.7-3.3	18,700	2/4
	1.7-3.3	37,400	4/4
	1.7-3.3	56,100	4/4
1.0% DRC-6749/ETOH ²	0-1.1	18,700	2/5
	0-1.1	37,400	3/5

¹ All percentages are v/v.

² From stock solutions containing 50% v/v DRC-6749 in 95% ethanol.

Table 6. Effect of cold temperatures (1.1-2.2°C) on male red-winged blackbirds sprayed with aqueous 10% v/v wetting agent solutions. All treatment groups sprayed with 0.25 cm water within 5 min of wetting agent treatment.

Solution	Rate (l/ha)	Mortality (deaths/N)
control (water only)	7,500	0/5
10% PA-14	470 935	2/5 5/5
10% DRC-6749/ETOH ¹	5,600 7,500	4/5 3/5

¹ From stock solution containing 50% v/v DRC-6749 in 95% ethanol.

Table 7. Effects of cold temperature (4.4°C) on male common grackles sprayed with aqueous 10% v/v wetting agent solutions. All treatment groups sprayed with 0.25 cm water within 5 min of wetting agent treatment¹, then another 0.25 cm water after approximately 3 h of cold exposure.

Solution	Rate (l/ha)	Mortality (dead/N)	
		<3h	total
control (water only)	3,740	0/5	0/5
10% PA-14	470	2/5	4/5
	935	2/5	3/5
10% DRC-6749	1,870	0/5	0/5
	3,740	0/5	0/5
10% DRC-6749/ETOH ¹	1,870	1/5	1/5
	3,740	1/5	1/5

¹ From stock solution containing 50% v/v DRC-6749 in 95% ethanol.

polymers, polyols, or poloxamers (Cosmetic, Toiletry, and Fragrance Assoc. designation). The manufacturer describes its line of polyols as "Virtually non-toxic..." (BASF Wyandotte Corp. 1973) and "...apparently free from hazardous toxicological or irritating properties..." (BASF Wyandotte Corp. 1982). Tests of related polyols on a bacterium, an alga, *Paramecium*, *Daphnia*, and *Lebistes* (a fish) led to the conclusion that "...examined... surfactants of (this) type cannot be classified among toxic compounds..." (Karpinska-Smulikowska 1984).

The toxicity of these compounds is reported to decrease predictably as molecular weight increases (Leaf 1967), and thus drop "...from 'slightly toxic'

to 'no effect'" (BASF Wyandotte 1973). At the same time, palatability decreases as molecular weight decreases (Leaf 1967), so that food treated with the relatively more toxic members of the group tends to be shunned, further enhancing safety. Thus animals dying in tests of oral toxicity succumb to inanition rather than to direct effects of the polyol tested (BASF Wyandotte 1982). Median lethal oral doses of a wide range of polyols ranged from about 5 to almost 35 g/kg for rats (Leaf 1967).

Some of these polyols are widely used in drug and cosmetic formulations. In fact, "It has been reported that the block copolymers are utilized in formulations which go into every cavity of the human body." (Schmolka 1967).

Table 8. Effects of cold temperature (1.1°C) on male red-winged blackbirds sprayed with aqueous wetting agent solutions followed by water applications.

Solution ¹	Rate (l/ha)	Water (cm)	Mortality (deaths/N)
control (water only)	5,600	0.76	0/5
10% DRC-6749	5,600	0.25	3/5
	5,600	0.76	5/5
20% DRC-6749	2,800	0.25	1/4
	2,800	0.76	5/5

¹ DRC-6749 solutions from stock solution containing 50% DRC-6749 in 95% ethanol. All percentages v/v.

Table 9. Species composition of bird kill resulting from roost treatment with DRC-6749, London, Ky., 21-22 February 1986.

Species	Estimated Roost Composition ¹ (%)	Composition of kill ² (%)
Common grackle	56	63
European starling (<i>Sturnus vulgaris</i>)	25	27
Red-winged blackbird	14	7
Brown-headed cowbird (<i>Molothrus ater</i>)	5	3
Rusty blackbird (<i>Euphagus carolinus</i>)	--	trace

¹ Estimated from incoming flights.

² From counted plots.

Human medical uses include employment as an emulsifier and stabilizer for injected emulsions (BASF Wyandotte 1982), and as an emulsifying agent in a fluorocarbon blood substitute (BASF Wyandotte 1973). DRC-6749 has been recommended as a wetting agent for cosmetics, a soap dispersant for soap bars and shampoos (Schmolka 1967), and a spreading agent in lotions (Schmolka 1974). Not many block copolymers have been tested for biodegradability, however this characteristic also appears to be predictable, decreasing with lower molecular weight and higher ethylene oxide content. Those tested seem to be slowly and incompletely biodegradable (Pawlaczik-Szpilowa et al. 1977). One block copolymer closely related to DRC-6749 has been described as "...essentially non biodegradable..." (R. Heffner, BASF Wyandotte Corp., pers. com-

mun.). This is not anticipated to be a significant problem, given the limited use to be expected of avian wetting agents, as long as tests continue to confirm little or no environmental hazard.

The field trial results were promising. Although the birds did not seem to be affected as rapidly as when PA-14 is used, indications were that most of the sprayed birds died. The bulk of the birds leaving the roost the morning after the spray operation left from the north side where wind had prevented the spray from reaching. The kill density of 252,000 birds/ha was only slightly under the average of 270,000 dead birds/ha for 14 prior PA-14 sprinkler system treatments (unpublished data). DRC-6749 was applied at a rate 2.5 times that at which PA-14 is normally used. Since DRC-6749 is somewhat more expensive than PA-14 (\$531.00/208 l drum of DRC-6749 compared with \$400.20/208 l drum of PA-14--1986 prices), indications are that chemical costs of a DRC-6749 operation may be at least 3 times that of a similar PA-14 operation. This may be acceptable in light of the greatly reduced aquatic hazards. Finally, the species composition comparisons (Table 9) do not seem to show any pronounced differential mortality among the species affected.

The use of alcohol as a mixing aid greatly simplifies dissolving DRC-6749 in water for application. As shown in Table 3, aquatic toxicities of ethanol and methanol appear to be low, if the rainbow trout is assumed to be a reliable indicator. No data were found on the aquatic toxicity of 2-propanol, but its use with PA-14 has not been reported to cause environmental problems. Until more information is generated on alcohol toxicities, ethanol would appear to be the cosolvent of choice for field applications of DRC-6749.

Results of research thus far indicate that DRC-6749 may satisfy the need for an effective wetting agent that can be used on birds roosting in wet areas, possibly even over water supporting aquatic life. Combined with simplified application techniques, e.g., large irrigation sweep sprinklers, or "water

cannons", it could be a widely useful tool in blackbird-starling roost management.

Additional studies will be necessary to confirm environmental safety and refine operational parameters before EPA registration is sought. These studies include:

- acute and chronic toxicity to benthic grazers such as snails and crawfish,
- chronic toxicity to fish and invertebrates, especially molluscs,
- a Daphnia life cycle test,
- oral and dermal mammalian toxicity,
- biodegradability in static and flowing water,
- phytotoxicity to typical roost vegetation,
- adjuvants and mixing techniques,
- volume and concentration for optimum wetting,
- confirmation of acceptably low alcohol toxicity.

Costs of conducting these studies, and possibly others, will have to be weighed against the long term benefits to be gained if DRC-6749 could be used for wetting agent treatment of aquatic roost sites.

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